

DOI: <https://doi.org/10.63359/z8jtmq52>

Evaluation of Bioaccumulation of Copper and Zinc In Soil and Trees of *Eucalyptus gomphocephala* and *Ficus nitida* in Darna City, Libya

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ARTICLE INFO

Vol. 7 No. 2 Agustus , 2025
Pages (72- 80)

Article history:

Revised form 07 July 2025
Accepted 31 July 2025

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Keywords:

Zinc, Copper, Soil, Leaves,
Eucalyptus gomphocephala ,
Ficus nitida

ABSTRACT

The study aimed to evaluate the bioaccumulation of heavy metals (copper & zinc) in the soil and leaves of *Eucalyptus gomphocephala* and *Ficus nitida* in three different areas in Darna city and feasibility of exploit it on biomonitoring of polluted air with heavy metals in the urban areas. The selected three areas were City centre, cement factory and steam station. complete randomized design was used in the experiment with three factors. The results showed significant differences among the study areas in their content of copper and zinc where city centre area recorded the highest zinc (Zn) content, followed by steam station area while the area of cement factory recorded the lowest accumulated content of zinc. The results showed that steam station and cement factory recorded higher accumulated content of copper(Cu) than the city centre which recorded the lowest content. The trees showed significant differences between them in their content of zinc, *Eucalyptus gomphocephala* recorded zinc content significantly higher than *Ficus nitida* while their copper content was in significant. The results also revealed high significant divergences between the samples (leaves and the soil) where zinc and copper content was significantly higher in the soil compared to the leaves, the highest copper content was in the soil of *Eucalyptus* tree at a rate of (1.395 mg/L) and in the soil of the steam station. *Eucalyptus* trees in the city centre recorded the maximum zinc content (12.75 mg/L). the effect of interaction between trees and samples types on content of zinc was insignificant whilst it was high significant on the content of copper. The highest zinc content was recorded in soil of *Eucalyptus* in the city centre at a rate of (14.157 mg/L), while the lowest zinc content was recorded in the soil of *Ficus* tree in the cement factory area at a rate of (10.493 mg/L).

تقدير التراكم الحيوي للنحاس والزنك في أشجار وترب الكافور *Eucalyptus*

gomphocephala والفيكس *Ficus nitida* في مدينة درنة - ليبيا

ز عطوط، مسعود مصطفى ، بوغرسة، صالح عطية ، جبريل ، سالمة إسماعيل

هدفت الدراسة إلى تقدير التراكم الحيوي للمعادن الثقيلة (النحاس والزنك) في ترب وأوراق الكافور *Eucalyptus gomphocephala* والفيكس *Ficus nitida* في ثلاث مواقع مختلفة في مدينة درنة وجدوى إمكانية استخدامها في الرصد الحيوي للهواء الملوث بالمعادن الثقيلة في المناطق الحضرية . المناطق التي تم اختيارها هي وسط المدينة ، مصنع الاسمنت و المحطة البخارية . استخدم التصميم كامل العشوائية في التجربة بثلاث عوامل . أظهرت النتائج اختلافات معنوية مابين مناطق الدراسة في محتواها من النحاس والزنك حيث سجلت وسط المدينة أعلى محتوى من الزنك متبوعا بمنطقة المحطة البخارية بينما سجلت منطقة

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مصنع الاسمنت أقل محتوى تراكمي من الزنك . بينت النتائج أن المحطة البخارية ومصنع الاسمنت سجلت اعلي محتوى تراكمي من النحاس مقارنة بوسط المدينة والتي سجلت أقل محتوى من النحاس . أظهرت الأشجار اختلافات معنوية في محتواها من الزنك حيث سجلت أشجار الكافور *Eucalyptus gomphocephala* أعلى محتوى من الزنك مقارنة بأشجار الفيكس *Ficus nitida* ، بينما لم يختلف معنويًا محتواها من النحاس . كشفت النتائج أيضا عن اختلافات عالية المعنوية ما بين عينات الأوراق والترب ، حيث كان محتوى الترب من النحاس والزنك أعلى من الأوراق . أعلى محتوى من النحاس تم تسجيله في تربة أشجار الكافور *Eucalyptus* بمعدل (1.395 مجم/لتر) وتربة المحطة البخارية . سجلت أشجار الكافور *Eucalyptus* في وسط المدينة أعلى محتوى من الزنك (12.75 مجم/لتر). تأثير التداخل ما بين الأشجار وأنواع العينات على محتوى الزنك لم يكن معنويًا بينما كان له تأثير عالي المعنوية على محتوى النحاس . تم تسجيل أعلى محتوى من الزنك في ترب أشجار الكافور في وسط المدينة بمعدل (14.157 مجم/لتر) ، بينما أقل محتوى من الزنك تم رصده في ترب أشجار الفيكس *Ficus* في منطقة مصنع الاسمنت بمعدل (10.493 مجم/لتر)

INTRODUCTION

In the last decades, the heavy metals are considering one of the most important inorganic pollutants to the environment and human health (Osman *et al.*, 2017). Heavy metals exist naturally in the soil as a result of rocks weathering (Kabata and Pendias, 2010). Emitted heavy metals from industrial and agricultural activities in addition to automobile exhaust are consider substantial pollution resources of air, the soil and the plants in the city centre along the traffic roads in addition to the factories adjacent areas (Ghrefat and Yusuf, 2006; Kelepertzis, 2014; Su *et al.*, 2014; Tóth *et al.*, 2016). Heavy metals like copper and zinc are essential nutrients for plants growth but on the other hand it becomes toxic when it turns into large quantities in the soil (Memon *et al.*, 2016). The toxicity of copper and zinc is commonly occurring whether by the air, food or drinking water (Anant *et al.*, 2018) exclusively if it included in the food chain (Arbaoui *et al.*, 2014) in certain times it interferes with metabolic processes (Jaishankar *et al.*, 2014). Conversely, the heavy metals like copper, zinc, iron and manganese play vital role in the nature since it is necessary for normal plants growth (Zhuang *et al.*, 2009). Zinc plays crucial role as a catalyst in many physiological processes (Cakmak, 2000) also copper plays essential role as co-enzyme and it considers one of the most important plants nutrients (Mahmood and Islam, 2006; Nagajyoti *et al.*, 2010). Nevertheless, the excessive accumulation of heavy metals might have toxic effect for the most plants. Symptoms of toxicity include necrosis, aging, wilting, atrophy and death (Kumar and Aery, 2016). Accumulation of zinc at a higher concentration than 300mcg⁻¹/ dry matter, it causes physiological alteration and inhibits growth (Foy *et al.*, 2003) it also inhibits many metabolic functions and leads to stunted growth, the appearance of aging signs, zinc toxicity also restricts the growth of roots and shoots (Fontes and Cox, 1998). In regard to human health, excess quantity of zinc leads to malfunction, poor growth and infertile (Nolan, 2003). Increased copper leads to or causes plant toxicity (Keller *et al.*, 2015). Polluted soil with heavy metals is considered a major issue and leads to adverse impact on soil characteristics and environmental functions (Castaldi *et al.*, 2004). Presence of excess copper in the soil induces cells toxicity, creates stress, plant damage and delayed

plant growth (Adrees *et al.*, 2015). Soil pollution is being increased over the long term, as a result of increase absorption and accumulation in the plant through the roots, bark, leaves and stems, where the most of the heavy metals being accumulated on the soil surface (Yargholi *et al.*, 2008). Number of different plants types have developed its capacity to endure the high levels of heavy metals and accumulation of higher concentrations (Ernst *et al.*, 1992). Endurance of Plant to heavy metals accumulation is considered a complicated phenomenon where these elements move via the roots and transfer to shoot system (Lombi *et al.*, 2002). Monitoring of heavy metals concentration in the environment is important matter since it could affect the human health (Sarwar *et al.*, 2017). Therefore, phytoremediation is safety technique by use the plants to recover and purify the polluted soils with heavy metals (Macnair, 2003; Salt *et al.*, 1998; Gratão *et al.*, 2005). Efficiency of phytoremediation differs depending on plant species, physiological and anatomical characteristics which affect on mechanisms of ions absorption (Osman *et al.*, 2017). In phytoremediation Technique, the plants characterize with rapid growth and high biomass to eliminate the heavy metals from surrounding environment (Chandra and Kumar, 2018). It depends on botanical extraction, botanical analysis, botanical installation, volatility and roots infiltration (Jadia and Fulekar, 2009). Therefore, estimation of bioaccumulation of heavy metals in the plants is so important as bio indicators of plants capacity to absorb the heavy metals. Accordingly, remediation by the plants is promising technique to clean the polluted areas by extract the heavy metals from the soil and accumulate it in the roots, stems and shoots (Arbaoui *et al.*, 2014; Osman *et al.*, 2017). Some plants being able to collect the heavy metals that have not certain biological functions (Baker and Brooks, 1989). Phytoremediation technique has been appeared to mitigate the pollution of heavy metals and organical chemical pollution on damaged areas by utilize the green plants (Chandra and Kumar, 2018).

MATERIALS AND METHODS

Study Area:

Three different locations were elected in Darna city:

- 1- the city centre
- 2- Area adjacent to the cement factory(east of the city)
- 3- steam station(city's western entrance) (Fig.1)



Figure (1): study areas Types of Trees: Two types of trees are selected

Eucalyptus gomphocephala : It is exotic tree which has planted widely in Al-gabal Al-Alakhdar area since thirties of the past century for soil erosion resistance on the roads sides and also as shade tree (Zunni and Bayoumi, 2006).

Ficus nitida: It has planted on large scale over the world (El-Etre and El-Tantawy, 2006).

Sampling Methods and Analysis

To estimate the bioaccumulation of heavy metals (copper & zinc) in the soil and leaves of *Ficus* and *Eucalyptus* trees, Leaf and soil samples were collected on November 26, 2021, and three replicates were taken from *Ficus* and *Eucalyptus* leaves as well as from the soil, Leaf samples were taken from four sides of each of both trees types. Soil were also collected from four sides of each replicate at a depth of 0-20 cm and mixed together to form a representative sample. Samples were packed in sealed and numbered nylon bags and transported to the laboratory for element measurements using the method of (Rowell, 1997; Lin *et al.*, 2003; Liu *et al.*, 2008). Total concentrations of heavy metals (Zn & Cu) were measured by wet digestion according to (Page *et al.*, 1982). The total concentration of heavy metals in the soil was estimated, and the concentration of each element was measured by Atomic Absorption Spectroscopy (AAS) at specific wavelengths, (324.8 nm) for copper and (213.9 nm) for zinc.

Statistical Analysis

The factorial experiment was analyzed with a three factor CRD design for the study using SAS software, version

2008, Duncans multiple range tests were used to compare the means of coefficients.

RESULTS AND DISCUSSION

Zinc, Copper and Boron are essential elements for plants, zinc plays crucial role on enzymes stimulation which assist growth and development of plants, zinc shortage may cause leaves distortion, copper has a crucial role on respiration process and creation of proteins and enzymes, insufficient quantity of copper may also result in leaves malformation and undergrowth, on the other hand, their high quantity bring about plant toxicity (Kabata, 2001). On the natural conditions, copper is one of the micro nutrients for several plants (Padmavathiamma and Li, 2007; Serbula *et al.*, 2012). Natural concentrations of copper in plants range from 3 to 30 mg/kg (Kabata, 2001), while the toxic concentrations range from 20 to 100 mg/kg (Padmavathiamma and Li, 2007), furthermore, high copper concentration causes delay of plant growth (Lewis *et al.*, 2001). Zinc also considers to be one of the crucial micro elements which participate on several photosynthesis aspects (Hu *et al.*, 2014), natural zinc concentration in plants ranges from 10 to 150 mg/kg. (Padmavathiamma and Li, 2007). In unfertilized and uncontaminated soil, the content of Zn ranges from 10 to 300 mg/kg (overall mean of around 50–55 mg/kg) (Barber,1995)

Table (1) shows the effect of study areas (city centre, cement factory, steam station) and tree type (eucalyptus & ficus) on leaf and soil copper and zinc content. The study showed that there are significant differences between the study areas in their zinc content where zinc concentration in the city centre was (12.322mg/L) followed by the steam station area with a concentration of (11.885 mg/L), then the cement factory area with the lowest content (11.350 mg/L). There are no significant differences between the steam station area and the cement factory area for copper accumulation (1.330, 1.249 mg/L) respectively, while the city centre area recorded the lowest copper content (1.150 mg/L). (Asaad *et al.*,2014) mentioned in their study of the heavy elements accumulation such as zinc and copper in the leaves of trees on the Syrian coast that it was high in all industrial areas such as power stations, cement factories and traffic areas. In contrast, (Schuhmacher *et al.*,2009) reported that cement production is an important source of heavy metal emissions such as copper and zinc, and that winds disperse cement dust over large areas (Thambavani and Kumar, 2011), heavy metal emissions from desalination factories are mainly due to mineral corrosion containing copper and zinc (Tularam and Ilahee, 2007; Lattemann and Höpner, 2008). High concentrations of metals such as zinc and copper in roadside soils in the cities are due to traffic emissions (Azeez *et al.*, 2014). Aslam *et al.*,2013) reported that copper and zinc in soil are within

the normal range and are not related to road traffic intensity. The majority of the heavy metals are toxic to the living organism and even those considered as essential can be toxic if present in excess. The heavy metals can impair important biochemical process posing a threat to human health, plant growth and animal life (Jarup, 2003)

As for type of trees, the results in Table (1) show significant differences between the *Ficus* and *Eucalyptus* trees in zinc content. Zinc concentration in *Eucalyptus* was (12.108 mg/L) compared to *Ficus* trees, which recorded (11.596 mg/L). The percentage of zinc accumulation in *Eucalyptus* trees compared to *Ficus* trees was approximately 4.41%. On the other hand, despite the slight increase in copper accumulation in *Eucalyptus* trees in comparison with *Ficus* trees, it was insignificant and ineffective. (Sarhan *et al.*, 2019) evaluated air pollution using *Eucalyptus globules* and *Ficus nitida* in Egypt, they noted that the increase in metals was due to uncontrolled disposal of industrial and domestic wastes, in addition, the response of *Ficus nitida* to metals was found to be higher than of *Eucalyptus globules*. In another study in Egypt, (El-khatib *et al.*,2020) tested the copper tolerance and physiological changes in *Eucalyptus* trees and *Ficus nitida*, they found that heavy metals can inhibit plant growth, they also noted that *Eucalyptus* has a better tolerance to copper than *Ficus*. They concluded that the ability of *Eucalyptus* to accumulate and tolerate heavy metals makes this species a good candidate for treating heavy metal pollution.

Regarding the sample type, the results showed that there were highly significant differences between leaves and soil in their copper and zinc content, as the concentration of copper and zinc in the soil was at rate of (1.328 mg/L, 11.911 mg/L) respectively, compared to the leaves that recorded the lowest content of copper and zinc at a rate of (1.168 mg/L, 11.793 mg/L) consecutively. The percentage of copper accumulation in the soil compared to the leaves was roughly 13.70% while the percentage difference between zinc accumulation in the soil and leaves was about 1.0%.

The results also showed that the effect of the interaction between the study area and the type of trees was insignificant for copper concentration, while it was highly significant for the zinc concentration. As for the effect of the interaction between the type of trees and the type of sample, it was insignificant for the zinc content, while it was significant for the copper content, the effect of the interaction between the study areas and the sample type in addition to the interaction effect of three factors were highly significant for the copper and zinc content. (Nasser *et al.*, 2022) estimated the concentration of heavy metals such as copper and zinc in *Ficus nitida* in Syria, where they found that the value of zinc was within the normal limits, unlike copper, the cumulative recording was (8.34), which supports the proposal to adopt *Ficus nitida* as a bioaccumulator to estimate pollution resulting from heavy elements. The copper and zinc contents in their

study were much more than those mentioned in the study of (Sahli and Belhiouni ,2021) on the leaves of *Ficus* trees planted in the city of Constantinople in Algeria.

Table (1): Effect of study areas, trees type and sample type on content of leaves and soil of copper and zinc (mg/L)

Treatments	Copper (Mg/L)	Zinc (Mg/L)
A)Areas		
City centre	1.150 ^b ±0.05 6	12.322 ^a ±0.20 0
Cement Factory	1.249 ^a ±0.00 6	11.350 ^c ±0.12 0
Steam station	1.330 ^a ±0.03 5	11.885 ^b ±0.07 3
Duncans	0.08668	0.07080
Sig.	0.001***	0.001***
B) Tree Type		
<i>Ficus nitida</i>	1.235 ^a ±0.00 9	11.596 ^b ±0.09 3
<i>Eucalyptusgomphocephala</i>	1.252 ^a ±0.04 5	12.108 ^a ±0.14 5
Sig.	0.637 ns	0.0001***
C) Sample Type		
Leaves	1.168 ^b ±0.03 4	11.793 ^b ±0.06 9
Soil	1.318 ^a ±0.02 7	11.911 ^a ±0.16 5
Sig.	0.001***	0.0001***
(A×B)	0.047 ns	0.0001***
(B×C)	0.0001***	0.240 ns
(A×C)	0.001***	0.0001***
(A×B×C)	0.002**	0.0001***
Standard Error(SE) ±		

The results in table (2) show that the highest copper content was in the soil of *Eucalyptus* tree at a rate of (1.395 mg/L), while the lowest copper content was in the leaves of *Eucalyptus* at a rate of (1.108 mg/L), copper content in the leaves and soil of *Ficus* tree was insignificant at a rate of (1.241,1.228) in the soil and leaves respectively. It is noted the accumulated copper(Cu) in the soil of eucalyptus trees is higher than that being accumulated in the same plant. This indicates the inability of eucalyptus roots to absorb copper and transfer it to the leaves. On the contrary, it was noted that the accumulation of copper is somewhat equal in the soil and leaves of ficus trees. This is due to the difference in plant species depending on their physiological and anatomical characteristics in addition to the roots infiltration which effect on mechanisms of ions absorption (Osman *et al.*, 2017).

Table (2): Effect of interaction between tree type and sample type on copper content (Cu)

Tree type Sample type	<i>Eucalyptus gomphocephalla</i>	<i>Ficus nitida</i>
Leaves	1.108 ^b	1.228 ^{ab}

Soil	1.395 ^a	1.241 ^{ab}
Duncans	***0.1734	

The results in Table (3) show the effect of the interaction between the study area and the sample type on the copper (Cu) content. The results showed that there is a significant divergence between the leaves and the soil of the city centre and steam station while copper content in the soil and leaves of cement factory was insignificant. The highest copper content was recorded in the soil of the steam station (1.405 mg/L), while the lowest of copper content was recorded in the leaves of city centre area (0.99 mg/L). Although there are no significant differences between the soils of the study areas in their copper content, the cumulative amount of copper in the soil of the city centre was higher that is recorded in the leaves compared to the copper content in the soil and leaves of cement factory and steam station.

Table (3): Effect of interaction between the study area and the sample type on the copper (Cu) content

Sample type \ Area	City centre	Cement factory	Steam station
	Leaves	0.990 ^b	1.258 ^a
Soil	1.309 ^a	1.241 ^a	1.405 ^a
Duncans	0.2123 ***		

The results in Table (4) indicated the effect of the interaction between the study area, tree type and sample type on the copper content. The results showed that the soil of *Eucalyptus* tree in steam station area recorded the highest copper content (1.553mg/L), followed by the *Eucalyptus* tree soil in the city centre area (1.436 mg/L), while the *Ficus* tree soil in the city centre area recorded the lowest copper content (1.181mg/L). On the other hand, the *Ficus* leaves in the cement factory area recorded the highest copper content (1.276 mg/L) followed by the leaves of the *Ficus* tree in the steam station area (1.261 mg/L), while the least copper content (0.883mg/L) was recorded in *Eucalyptus* leaves in the city centre.

Table (4): Effect of the interaction between the study area, tree type and sample type on the copper(Cu) content

Area		City centre		Cement factory		Steam station	
Tree type		Eucalyptus	Ficus	Eucalyptus	Ficus	Eucalyptus	Ficus
Sample type	Leaves	0.833 ^b	1.148 ^b	1.240 ^b	1.276 ^{ab}	1.250 ^b	1.261 ^{ab}
	Soil	1.436 ^a	1.181 ^b	1.197 ^b	1.284 ^a	1.553 ^a	1.257 ^{ab}
Duncans		**0.3003					

The findings in Table (5) illustrate significant differences between the study area and tree type , as it clear that *Eucalyptus* trees in the city centre recorded the maximum zinc content (12.75 mg/L) , and *Ficus* trees in the cement factory recorded the lowest zinc content (10.92 mg/L).

Table (5): Effect of interaction between the study area and tree type on zinc (Zn) content

Tree type \ Area	City centre	Cement factory	Steam station
<i>Ficus nitida</i>	11.898 ^{bc}	10.92 ^d	11.97 ^b
<i>Eucalyptus gomphocephalla</i>	12.75 ^a	11.78 ^c	11.80 ^{bc}
Duncans	***0.1734		

The results in Table (6) showed significant differences between the study area and the type of sample in both leaves and soil, as the soil of city centre recorded the highest zinc content rate at (13.36 mg/L), while the lowest zinc content was obtained in the cement factory soil at (10.828 mg/L).

Table (6): Effect of interaction between study area and sample type on zinc (Zn) content

Sample type \ Area	City centre	Cement factory	Steam station
Leaves	11.283 ^c	11.872 ^c	12.225 ^b
Soil	13.360 ^a	10.828 ^f	11.545 ^d
Duncans	***0.1734		

The results in Table (7) showed significant divergences between the study area, tree type and sample type on zinc (Zn) content. The highest zinc content was achieved in soil of *Eucalyptus* in the city centre at a rate of (14.157 mg/L), while the lowest zinc content was recorded in the soil of *Ficus* tree in the cement factory area at a rate of (10.493 mg/L). Comparison of leaf zinc content across the study areas, *Eucalyptus*leaves in the cement factory recorded the maximum cumulative content (12.390 mg/L) with a slight insignificant increase over the *Eucalyptus* leaves in the steam station area (12.373 mg/L) , while the least content of zinc was recorded in *Ficus* leaves in the city centre at a rate of (11.233 mg/L) .

Table (7): Effect of interaction between study area, tree type and sample type on zinc (Zn) content

Area		City centre		Cement factory		Steam station	
Tree type		Eucalyptus	Ficus	Eucalyptus	Ficus	Eucalyptus	Ficus
Sample Type	Leaves	11.333d	11.233d	12.390b	11.353d	12.373b	12.077c
	Soil	14.157a	12.563b	11.163d	10.493e	11.233d	11.857c
Duncans		***0.2453					

Conclusion:

The results showed significant differences between the study area in their copper and zinc content. The city centre area recorded the highest zinc content, followed by the steam station which at the same time recorded the highest copper content succeeded by the cement factory area. *Eucalyptus* trees recorded significantly the highest zinc accumulation compared with *Ficus* tree. Insignificant differences appeared between them in their copper content. The sample type also recorded highly significant differences where soil recorded significantly the highest copper and zinc content. Therefore, the current study recommends increasing the green belt area, such as *Ficus nitida* and *Eucalyptus gomphocephala* around industrial and urban areas for their effective role in absorbing heavy pollutants and purifying the air and the environment, Phytoremediation has emerged as the method of choice for cleaning up a broad range of environmental pollutants. This solar-driven, eco-friendly, green technology is often favoured over more conventional methods of clean-up due to its low cost, low impact, and wider public acceptance.

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